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APPLICATION NUMBER: 60/492,402

FILING DATE: August 04, 2003

RELATED PCT APPLICATION NUMBER: PCT/US04/24611

Certified by



Jon W Dudas

Acting Under Secretary of Commerce
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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

19704 U.S. PTO
60749242

08/04/03

INVENTOR(S)					
Given Name (first and middle (if any))		Family Name or Surname		Residence (City and either State or Foreign Country)	
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<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
A NOVEL PHARMACOLOGICAL PATHWAY DESTABILIZES LYSOSOMES AND TARGETS ONCOGENIC OR ABERRANT PROTEINS FOR DESTRUCTION					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
<input checked="" type="checkbox"/> Customer Number		26259		<div>Place Customer Number Bar Code Label here</div>	
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification		Number of Pages		4	
<input type="checkbox"/> Drawing(s)		Number of Sheets		<input type="checkbox"/> CD(s), Number	
<input type="checkbox"/> Application Data Sheet. See 37 CFR 1.76				<input checked="" type="checkbox"/> Other (specify)	
				Return Postcard	
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27.				FILING FEE AMOUNT (\$)	
<input type="checkbox"/> A check or money order is enclosed to cover the filing fees					
<input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number		50-1619		\$80.00	
<input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached.					
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input type="checkbox"/> No.					
<input checked="" type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: NIH R01-CA72275					

Respectfully submitted,

Date 8/4/03

SIGNATURE 

TYPED or PRINTED NAME Jane Massey Licata

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(if appropriate)

Docket Number: DC-0235

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

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- 1) Provisional Patent Application Transmittal Letter
(Small Entity) (2 copies);
- 2) Application consisting of 4 pages of Specification,
including one (1) page of Claims;
- 3) Return Post Card; and
- 4) Authorization to charge deposit account in the amount of
\$80.00.



JANE MASSEY LICATA

**A Novel Pharmacological
5 Pathway Destabilizes Lysosomes and Targets Oncogenic
 or Aberrant Proteins for Destruction**

 This invention was supported in part by funds from the
10 U.S. government (NIH Grant No. RO1-CA62275) and the U.S.
government may therefore have certain rights in the
invention.

 This provisional patent application describes a novel
pharmacological pathway that destabilizes lysosomes and
15 targets oncogenic or other aberrant proteins for
destruction. This previously unrecognized mechanism was
uncovered in acute promyelocytic leukemia (APL) cells but
is also active in other cells. APL cases most often result
from the t(15;17) balanced chromosomal translocation, which
20 expresses the fusion product, *Pml/Rar alpha*. *Pml/Rar alpha*
can block differentiation of promyelocytes. Expression of
Pml/Rar alpha leads to accumulation in affected cases of
immature promyelocytes in the bone marrow and peripheral
blood. APL cases are typically sensitive to all-*trans*
25 retinoic acid (RA) treatment, which causes degradation of
Pml/Rar alpha overcoming the dominant-negative effects of
this translocation product. This degradation after RA-
treatment occurs through proteasome as well as caspase
dependent pathways. RA in turn triggers differentiation of
30 the leukemic promyelocytes. However, RA resistant APL
cases can occur and the trivalent form of arsenic (As⁺³) has
been used successfully in the treatment of these cases.
Arsenic also induces degradation of *Pml/Rar alpha*, but
mechanisms engaged in this degradation have not been well

understood and are distinct from those activated by RA-treatment. The findings reported here indicate that

5 arsenic can act through a previously unknown mechanism that targets *Pml/Rar alpha* for degradation by rapidly destabilizing lysosomes. Destabilization leads to release of lysosomal enzymes, which triggers *Pml/Rar alpha* degradation in NB4 APL cells. Arsenic was able to

10 destabilize rapidly lysosomes in these leukemic cells. This occurred within 2 hours of arsenic treatment as assessed in NB4 APL cells using a lysosome-specific targeting dye and confocal microscopy. Using SDS-PAGE and immunoblot analyses, lysosomal protein esterase was

15 detected in the cytosolic fraction of cultured APL cells as soon as 5 minutes after arsenic treatment and at dosages that are clinically achievable. Furthermore, lysosomal cathepsin B appeared in the cytosol within 30 minutes of arsenic treatment of APL cells while cytochrome c (an

20 activator of caspase-induced apoptosis) was not detected in the cytosol until 96 hours. Notably, proteasome or caspase inhibitors did not prevent this rapid arsenic-induced *Pml/Rar alpha* degradation, indicating that the mechanism engaged was novel. Consistent with this view, isolated

25 lysosomes from NB4 APL cells could reproduce this degradation *in vitro*. This arsenic activated degradation program was also engaged by non-leukemic cells and was able to destroy leukemogenic and other aberrant proteins since the cystic fibrosis protein (CFTR) was also sensitive to

30 this degradation. We conclude that this lysosome-dependent degradative pathway is novel. It was initially found in leukemic and non-leukemic cells following arsenic treatment. This therapeutic pathway can target *Pml/Rar alpha* and other aberrant proteins for destruction.

Furthermore, this activated lysosome-degradation pathway can also be used to screen for small molecule activators.

- 5 This screening would identify pharmacological agents in addition to arsenic that would have therapeutic activity via induced destabilization of lysosomes that would in turn eliminate oncogenic or other aberrant proteins by triggering their destruction. We propose that this new
- 10 lysosomal degradation pathway that is activated by arsenic or other compounds to be uncovered using this novel screen will have therapeutic activity. This will occur in clinical settings where the disease state depends on expression of oncogenic or aberrant proteins susceptible to
- 15 this degradation.

What is claimed is:

- 5 1. A method for identifying an agent which
destabilizes lysosomes to increase oncogenic or aberrant
protein degradation comprising contacting a lysosome or a
cell containing a lysosome with an agent and detecting
whether said agent destabilizes lysosomes thereby
10 increasing oncogenic or aberrant protein degradation.
2. An agent identified by the method of claim 1.
3. The method of claim 2, wherein the agent is
15 arsenic.
4. A method for destabilizing lysosomes comprising
administering to a lysosome or a cell containing a lysosome
an agent of claim 2 so that the lysosome is destabilized.
20
5. The method of claim 4, wherein the lysosome is
destabilized and an oncogenic or aberrant protein is
degraded.
- 25 6. A method for treating a disease or condition
associated with an oncogenic or aberrant protein comprising
administering an agent of claim 2.

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Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse